REMARKS

Claims 1-21 are all the claims pending in the application.

Claims 5-10 and 15-20 have been cancelled.

In paragraph No. 1, page 2 of the Office Action, the Examiner alleges that claims 3 and 4 are too broad.

Specifically, the Examiner alleges that a polypeptide in claim 3, having at least one deletion, addition, insertion or substitution of the polypeptide of SEQ ID NO:8 and having an activity to release saccharides from a disaccharide glycoside, was known in plants.

In response, applicants have amended claims 3 and 4 to recite a polypeptide "isolated from a microorganism."

In paragraph No. 2, page 2 of the Office Action, the Examiner alleges that the title of the invention is not descriptive.

In response, applicants have amended the title to recite "Glycosidase Isolated From Microorganisms."

In paragraph No. 3, page 3 of the Office Action, the Examiner objects to claims 1, 3, 11, and 13 because of the informalities.

Specifically, the Examiner alleges that the term "in disaccharide unit" is grammatically incorrect.

In response, applicants have amended the claims to recite "in a disaccharide unit."

In view of applicants' amendments, the Examiner is respectfully requested to reconsider and withdraw the objection to claims 1, 3, 11, and 13.

In paragraph No. 4, page 3 of the Office Action, the Examiner objects to claim 13 as reciting the subject matter of non elected claim 10.

In response, applicants have amended claim 13 to delete the reference to claim 10.

In view of applicants' amendment, the Examiner is respectfully requested to reconsider and withdraw the objection to claim 13.

In paragraph No. 5, page 3 of the Office Action, the Examiner rejects claims 1-4 under 35 U.S.C. § 101 as allegedly being directed to non-statutory subject matter.

Specifically, the Examiner alleges that the claims are drawn to enzymes and polypeptides and read on products of nature.

In response, applicants have amended the claims to recite an enzyme or a polypeptide "isolated from a microorganism."

In view of applicants' amendments, the Examiner is respectfully requested to reconsider and withdraw the rejection to claims 1-4.

In paragraph No. 6, page 3 of the Office Action, the Examiner rejects Claims 1-3 and 11-14 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite.

Regarding claim 1, the Examiner alleges that the recitation of "microorganism-derived enzyme" is unclear as to whether it means an enzyme "isolated from a microorganism" or that "produced by a microorganism."

In response, applicants have amended claim 1 to recite "an enzyme isolated from a microorganism."

In view of applicants' amendments, the Examiner is respectfully requested to reconsider and withdraw the rejection.

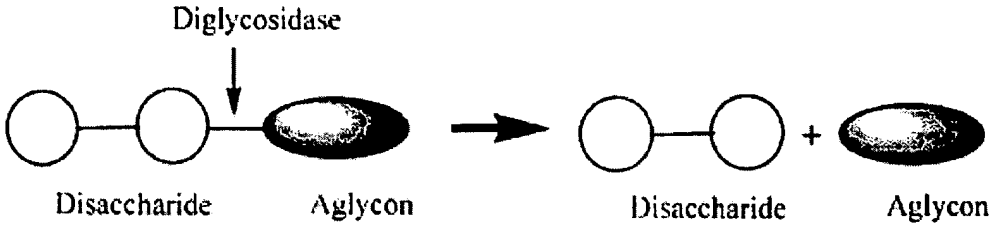
Regarding claims 1, 3, 11, and 13, the Examiner alleges that they are confusing in the recitation of "having an activity to act upon a disaccharide glycoside to release saccharides from said disaccharide glycoside in disaccharide unit."

Specifically, the Examiner asserts that, based on the specification at page 4, lines 3-9, it appears that the term means an enzymatic activity wherein a disaccharide is cleaved from a polysaccharide, i.e., diglycosidase activity. While, it appears from the specification at page 4, lines 9-16, the enzyme also has the activity of cleaving disaccharides into monosaccharides.

Applicants respectfully submit that the term is not indefinite. The function of the enzyme according to the present invention (i.e., having an activity to act upon a disaccharide glycoside to release saccharides from said disaccharide glycoside in disaccharide unit) can be explained by the figure shown below.

The enzyme of the present invention cuts the bonding (i.e., glycoside linkage) between an aglycon (non-saccharide moiety) and a saccharide. When a disaccharide glycoside is a substrate, the enzyme generates a disaccharide and an aglycon (non-saccharide moiety) by the enzymatic activity.

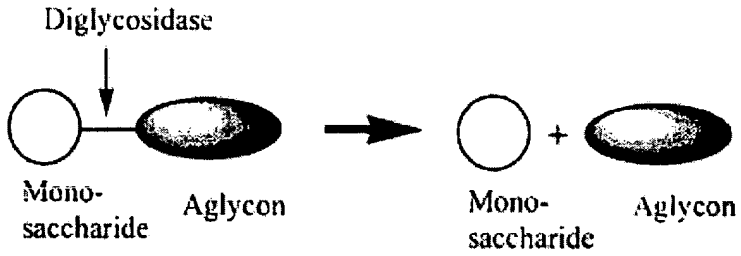
Function of the present enzyme on disaccharide glycoside:



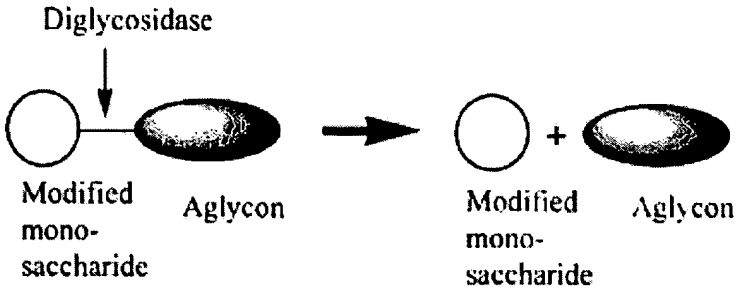
Such activity is defined in the present application. That is, the present enzyme acts upon a disaccharide from said disaccharide glycoside to release saccharides from said disaccharide glycoside in a disaccharide unit. (This functional mechanism is described in Examples 13, 14, 15 and 16.)

The description at page 4, lines 9-16 (The diglycosidase of the present invention has not only the activity to act on the disaccharide glycoside to release the saccharide in a disaccharide unit but also cut the glycoside bonding of the monoglycoside. Moreover it has an activity to cut the glycoside bonding of the modified monoglycoside.) is to mention that the present invention can act on monoglycosides and modified monoglycosides in addition to the activity on the above-explained disaccharides. These reactions are shown in the following figure.

Function of the present enzyme on monoglycoside:



Function of the present enzyme on modified monoglycoside:



In other words, even when a monoglycoside or a modified monoglycoside is a substrate, the present enzyme acts on the bonding (i.e., glycoside linkage) between an aglycon (non-saccharide moiety) and a saccharide to generate a monosaccharide and an aglycon (non-saccharide moiety) or a modified monosaccharide and an aglycon (non-saccharide moiety). This functional mechanism of the present enzyme on the modified glycoside is described in Example 11.

In view of the above, the Examiner is respectfully requested to reconsider and withdraw the rejection to claims 1, 3, 11, and 13.

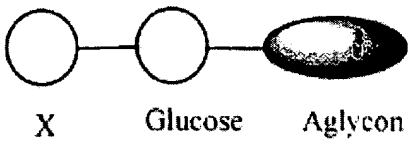
Regarding claim 2, the Examiner alleges that the recitation of "analogous disaccharide glycoside" renders the claim indefinite.

Specifically, the Examiner alleges that, although applicants have provided a definition for analogous disaccharide glycoside at the bottom of page 6 of the specification and examples of analogous disaccharide glycosides, it remains unclear as to how "analogous" to β-primeveroside a disaccharide glycoside must be to be included in the scope of the claim.

Applicants respectfully submit that the term "analogous disaccharide glycoside" is sufficiently defined.

The disaccharide glycoside which is analogous to primeveroside is a disaccharide glycoside having a glucose moiety in the aglycon (non-saccharide moiety) side. In the specification, apiofuranosyl-β-D-glucopyranoside and arabinofuranosyl-β-D-glucopyranoside are described as the examples of the disaccharide glycoside which is analogous to primeveroside.

The disaccharide glycoside which is analogous to primeveroside is shown by the following figure:



When it is primeveroside, X is xylose.

When it is apiofuranosyl-β-D-glucopyranoside, X is apiose.

When it is arabinofuranosyl-β-D-glucopyranoside, X is arabinose.

For further clarification, applicants have amended the specification, page 6, lines 1-3 from the bottom, to recite "are disaccharide glycosides having glucose on the aglycon side, such as apiofuranosyl-β-D-glucopyranoside and arabinofuranosyl-β-D-glucopyranoside."

In view of the above, the Examiner is respectfully requested to reconsider and withdraw the rejection to claim 2.

In paragraph No. 10, page 5 of the Office Action, the Examiner rejects Claims 1-3 and 11-14 under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification.

Specifically, the Examiner alleges that claims 1-3, 11 and 13 are directed to: a genus of microorganism-derived enzymes with diglycosidase activity (claim 1) and optionally wherein the enzyme releases a disaccharide from β-primeveroside and/or an analogous disaccharide glycoside (claim 2); a genus of polypeptides having at least one deletion, addition, insertion or substitution of the polypeptide of SEQ ID NO:8 and having diglycosidase activity (claim 3); a method of producing a genus of enzymes with diglycosidase activity by culturing a genus of microorganisms (claim 11) and optionally wherein the genus of microorganisms is selected from Aspergillus, Penicillium, Rhizopus, Rhizomucor, Talaromyces, Mortierella, Cryptococcus, Microbacterium, Corynebacterium, and Actinoplanes (claim 12), and optionally wherein the nutrient medium contains a genus of substances that induce production of an enzyme having diglycosidase activity (claim 13). The Examiner further asserts that the specification teaches the structure of only two representative species of such glycosidases, and fails to describe any other representative species by any identifying characteristics or properties other than the functionality. The Examiner concludes that the specification lacks description of representative species encompassed by the genus of the claims.

Regarding the genus diglycosidase, the enzyme of the present invention is characterized in that it has an activity to release saccharides in a disaccharide unit from the disaccharide glycoside by acting on the disaccharide glycoside which can hardly be utilized as the substrate

by the known glycosidase (page 4, lines 3-9). In addition to the substrate specificity, the present enzyme has the enzymological and chemical properties recited in the amended claims, i.e., the enzyme of the present invention has a substantial activity even at a pH 3 or less and is stable at 50°C or less.

In view of applicants' amendments, the Examiner is respectfully requested to reconsider and withdraw the rejection to claims 1-3.

Regarding the genus microorganism, applicants respectfully submit that the disclosure of the specification is sufficient to support a wide range of microorganisms.

Applicants have searched for the diglycosidase in a broad range of natural sources (pages 11-14), and found that screening using eugenylprimeveroside as the sole carbon source identified four (4) strains capable of showing the diglycosidase activity of the present invention. The first strain was Aspergillus fumigatus and the other 3 strains were Aspergillus niger. Further, as shown in Example 1 of the specification, the inventors succeeded in producing diglycosidase of the present invention from Aspergillus niger. In Example 2, a production example of the diglycosidase of the present invention by Aspergillus fumigatus is shown. In Example 5, it is shown that a method similar to the method for Aspergillus fumigatus and Aspergillus niger (culturing in a nutrient medium) is applicable to other microorganisms screened from nature, i.e., 21 types of cultured strains including mold, yeast, bacteria, and actinomycetes can produce the disaccharide glycoside of the present invention.

In view of the above, the Examiner is respectfully requested to reconsider and withdraw the rejection to claims 11 and 12.

Regarding the genus inducer, applicants have amended claim 13 to recite that the nutrient medium "is obtainable by adding a saccharide," and amended claim 14 to recite that the inducer is a saccharide "selected from the group consisting of gentose, gentiobiose, and gentio-oligosaccharide."

As explained in Example 3 in the specification, addition of a saccharide into the culturing medium for culturing *Aspergilus fumigatus* for producing a diglycosidase of the present invention showed an inducing effect for the production of the diglycosidase. Among various saccharides, gentiose, gentiobiose, gentio- and oligosaccharide showed remarkable effects. This induction effect was found for two strains of *Aspergillus niger* in addition to the *Aspergilus fumigatus*.

In view of applicants' amendments, the Examiner is respectfully requested to reconsider and withdraw the rejection to claims 13 and 14.

In paragraph No. 11, page 6 of the Office Action, the Examiner rejects Claims 1-3 and 11-14 under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement.

Specifically, the Examiner alleges that the specification does not reasonably provide enablement for *all* enzymes, *any* analogous disaccharide glycosides, *all* polypeptides, *any* microorganism, and *any* substance as presently claimed.

Applicants submit that this rejection should be withdrawn for the same reasons that the rejection stated in paragraph 10 of the Office Action should be withdrawn.

In paragraph No. 12, page 8 of the Office Action, the Examiner rejects Claims 1-3 and 11-14 under 35 U.S.C. § 102(b) as allegedly being anticipated by or, in the alternative, under 35 U.S.C. § 103(a) as allegedly obvious over McCormack et al.

Specifically, the Examiner alleges that McCormack et al. teach a method of producing an enzyme having chitobiase activity by culturing *Talaromyces emersonii* grown in a medium containing chitin for enzyme induction, and of isolating the enzyme. The Examiner concluded that McCormack et al. anticipate claims 1-3 and 11-14 as written.

In response, applicants respectfully submit that McCormack et al. do not teach the present invention or render it obvious.

McCormack et al. describe that chitobiase derived from *Talaromyces emersonii* (fungus) generates diacetylchitobiose (disaccharide) from chitin as a substrate (chitin is not a glycoside but is a polysaccharide having a straight-chain molecule in which D-glucosamines acetylated at the amino group are linked by β-1,4 bonding).

On the other hand, the diglycosidase of the present invention uses a glycoside as a substrate and acts upon the bonding site between an aglycon (non-saccharide moiety) and a saccharide chain to release saccharides in a disaccharide unit.

Thus, the enzyme of the present invention is clearly different from the enzyme of the reference in that the substrate is a glycoside.

In view of the above, the Examiner is respectfully requested to reconsider and withdraw the rejections.

In paragraph No. 13, page 9 of the Office Action, the Examiner rejects Claims 1-3, 11, 13, and 14 under 35 U.S.C. § 102(e) as allegedly being anticipated by or, in the alternative, under 35 U.S.C. § 103(a) as allegedly obvious over Harman et al.

Specifically, the Examiner alleges that Harman et al. teach an enzyme isolated from *Trichoderma harzianum* strain P1 having chitobiase activity, wherein the enzyme cleaves a dimeric unit from chitin and a method of producing the chitobiase enzyme in Modified Richard's medium. The Examiner concludes that one of ordinary skill in the art at the time of the invention would have recognized that the chitin was added to the Modified Richard's medium for chitin induction.

In response, applicants respectfully submit that Harman et al. do not teach the present invention or render it obvious.

Harman et al. describe that an enzyme which releases dimers from chitin was isolated from *Trichoderma harzianum.* This chitobiase is an enzyme which generates diacetylchitobiose (disaccharide) from chitin as a substrate (chitin is not a glycoside but is a polysaccharide having a straight-chain molecule in which D-glucosamines acetylated at the amino group are linked by β-1,4 bonding).

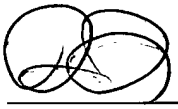
On the other hand, the diglycosidase of the present invention uses a glycoside as a substrate and releases saccharides in a disaccharide unit. Accordingly, the enzyme of the present invention is novel and unobvious from the enzyme of the reference which uses chitin (not a glycoside) as a substrate.

In view of the above, the Examiner is respectfully requested to reconsider and withdraw the rejections.

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,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Date: October 30, 2002

APPENDIX

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE TITLE:

The title is changed as follows:

~~Novel Enzyme Composition and Production Method and Use Thereof~~ Diglycosidase Isolated From Microorganisms

IN THE SPECIFICATION:

The specification is changed as follows:

Pages 69-70, Second paragraph:

A 10 μg portion of each of the thus obtained various chromosomal DNA preparations was digested with *Bam*HI in the case of *Aspergillus fumigatus, Aspergillus oryzae, Aspergillus niger, Aspergillus aculaetus, Penicillium lilacinum, Penicillium decumbence, Penicillium* ~~*multicolar*~~ *multicolor, Talaromyces emersonii, Mortierella vinacea, Cryptococcus albidus, Corynebacterium ammoniagenes, Corynebacterium glutamicum, Microbacterium arborescens* and *Penicillium rugolosum*, or with *Eco*RI in the case of *Rhizopus oryzae, Rhizomucor pusillus, Rhizomucor miehei* and *Actinoplanes missouriensis*, and the resulting digest was applied to a 1% agarose gel electrophoresis. As a control, the gene fragment of an enzyme similar to the tea-derived diglycosidase used as the probe was also subjected to the same gel electrophoresis. After the electrophoresis, DNA samples were blotted on a nylon membrane and hybridization was carried out using a labeled gene fragment p of an enzyme similar to the tea-derived diglycosidase (structural gene moiety of matured plant primeverosidase gene) as the probe, using DIG System

Kit (Boehringer Mannheim) in accordance with the instruction attached thereto. As a result, when the detection was carried out under hybridization conditions (5 x SSC, 1% blocking agent, 0.1% N-lauroylsarcosine sodium, 0.02% SDS, 68°C, overnight) and washing conditions (6 x SSC, 0.1% SDS, room temperature, 5 min. x 2 and 6 x SSC, 0.1% SDS, 45°C, 15 min. x 2), a signal was obtained at a position where the plant gene was blotted, but the signal was not observed at any other position where the microorganism-derived genome was blotted. Thus, it is considered that the microorganism-derived diglycosidase gene has a structure which is different from the plant primeverosidase gene.

Pages 70-71, Third paragraph:

In addition, it was able to detect the signal in *Aspergillus oryzae, Aspergillus niger, Aspergillus aculeatus, Penicillium* ~~*multicolar*~~ *multicolor, Penicillium lilacinum, Corynebacterium ammoniagenes* and *Corynebacterium glutamicum*, even under more stringent washing conditions (5 x SSC, room temperature, 10 min. and 4 x SSC, 68°C, 30 min.).

Page 72, First paragraph:

Each of acetylglycitin, acetylgenistin, acetyldaidzin, malonylglycitin, malonylgenistin and malonyldaidzin (produced by Fujicco, available from Nakalai Tesque) was allowed to react with a diglycosidase enzyme solution prepared from *Asp. fumigatus* or *Pen.* ~~*multicolar*~~ *multicolor* or with an almond-derived glucosidase (mfd. by Sigma) under the following conditions.

Page 6, Last paragraph:

In order to obtain a microorganism capable of producing an enzyme having a diglycosidase activity, the present inventors have examined a broad range of natural sources and found that several microbial strains isolated from the natural world can produce an enzyme having said activity. The disaccharide glycosides analogous to β-primeveroside are disaccharides glycosides having glucose on the aglycon side, such as apiofuranosyl-β-D-glucopyranoside and arabinofuranosyl-β-D-glucopyranoside.

IN THE CLAIMS:

Claims 5-10 and 15-20 are canceled.

The claims are amended as follows:

1. (amended) ~~A~~ An enzyme isolated from a microorganism ~~derived enzyme~~ having an activity to act upon a disaccharide glycoside to thereby release saccharides from said disaccharide glycoside in a disaccharide unit,

wherein said enzyme has a substantial activity even at a pH 3 or less and is stable at 50°C or less.

3. (amended) A polypeptide isolated from a microorganism which comprises a polypeptide having the amino acid sequence of SEQ ID NO: 8 shown in the Sequence Listing, wherein one or more amino acid residues of the amino acid sequence are modified by at least one of deletion, addition, insertion and substitution, and also having an activity to act upon a disaccharide glycoside to release saccharides from said disaccharide glycoside in a disaccharide unit.

4. (amended) A polypeptide isolated from a microorganism which comprises a polypeptide having the amino acid sequence of SEQ ID NO: 8 shown in the Sequence Listing.

11. (amended) A method for producing an enzyme having an activity to act upon a disaccharide glycoside to release saccharides from said disaccharide glycoside in a disaccharide unit, which comprises culturing a microorganisem in a nutrient medium to effect production of the enzyme having an activity to act upon a disaccharide glycoside to release saccharides from said disaccharide glycoside in a disaccharide unit, and subsequently collecting said enzyme from the resulting culture mixture,

wherein said enzyme has a substantial activity even at pH 3 or less and is stable at 50°C or less.

13. (amended) The method for producing an enzyme having an activity to act upon a disaccharide glycoside to release saccharides from said disaccharide glycoside in a disaccharide unit according to claim ~~10,~~ 11 or 12, wherein the ~~nutrient medium contains a substance which induces production of an enzyme having an action to release saccharides from a disaccharide glycoside in disaccharide unit~~ enzyme is inducible by addition of a saccharide to the nutrient medium.

14. (amended) The method for producing ~~a novel~~ an enzyme ~~composition~~ according to claim 13, wherein the ~~inducer is a~~ saccharide is selected from the group consisting of gentose, gentiobiose, and gentio-oligosaccharide.

Claim 21 is added as a new claim.